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Drug Combinations for Treatment of Mice Infected with Acyclovir-Resistant Herpes Simplex Virus

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The purpose of this study was to determine whether acyclovir resistance in mice infected with herpes simplex virus could be overcome by using high doses of acyclovir or vidarabine alone or by using a combination of the two drugs. The results indicate that the mice infected intracerebrally were refractory to acyclovir alone but responded to vidarabine or a combination of vidarabine and acyclovir. These observations have major implications for the clinical management of severe herpetic infections, particularly if attempts are to be made to devise means of circumventing drug resistance.

Acyclovir (ACV) is being used or is under investigation for use for various herpes simplex virus (HSV) infections. The widespread use of ACV raises important concerns regarding the possible increased appearance and transmission of ACV-resistant strains. ACV-resistant HSV variants can be isolated from patients either before or after repeated treatments with ACV (1, 8). Reports by our group (10a) and Swedish workers (15) indicate that in some instances the in vitro resistance found in isolates obtained from patients undergoing ACV treatment can be translated to clinical resistance. In these studies, the patients continued to have lesions and shed ACV-resistant virus of the thymidine kinase-deficient (TK^D) phenotype in spite of antiviral chemotherapy. The predominant phenotype of clinical ACV-resistant variants isolated by other investigators was also TK^D (1, 8), although a thymidine kinase-altered variant has also been reported (8; M. N. Ellis, P. M. Keller, S. E. Straus, S. Nusinoff-Lehrman, and D. Barry, Abstr. Ninth Int. Herpesvirus Workshop, p. 255, 1984).

Therefore, it appeared of potential clinical importance to ascertain whether ACV-resistant clones prepared in cell culture were still virulent in mice, and if so, whether high doses of ACV alone or in combination with vidarabine (ara-A) could still be an effective treatment. The results would be clinically relevant since it has been argued that in vivo resistance can be overcome by the use of a high dose of an antiviral drug and that a combined approach may not be necessary (Workshop on the Evaluation of Antiviral Drugs and Interferon in Herpesvirus Animal Models, National Institutes of Health, Bethesda, Md., May 16 to 17 1985). A combination of ACV and ara-A is being considered for the treatment of HSV encephalitis; compared with single-drug therapy, this approach may be more effective, less toxic, and more likely to inhibit or prevent the development of drug-resistant virus.

(Part of this work was presented at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, Nev., 24 to 26 October 1983 [abstr. no. 566, p. 187].)

Various ACV-resistant HSV type 2 (HSV-2) TK^D variants were prepared in our laboratory by single passage in the presence of 10 μM ACV, as described previously (11). We have concentrated on studying the resistance of HSV-2 clones because the parent strain (G) is about 100 to 10,000 times more virulent in mice than are several HSV type 1 (HSV-1) strains (4, 9, 13) and because earlier studies in animal models have primarily focused on HSV-1 variants (6, 7, 16, 17).

An example of such a clone, G-ACV-C1, was found in plaque assays (11) to be 40-fold more resistant to ACV than was the parental clone, to be still susceptible to ara-A in cell culture, and to be TK^D; this virus expressed less than 0.2% of the TK activity found in the parental virus, as determined in TK^D HeLa Bu 25 cells (3). The TK^D virus was first characterized for its ability to cause central nervous system disease in newborn and adult mice. G-ACV-C1 was about 164-fold less virulent than the parental clone (ACV-sensitive TK^+ virus) when inoculated intracerebrally in 6-week-old female ICR mice (50% lethal dose ≈ 655 PFU, as determined by the median effect method [10]) (Table 1). However, it was only about 39-fold less virulent to 7- to 10-day-old mice (50% lethal dose ≈156 PFU; data not shown), supporting the findings of Tenser (16) that newborn mice may be more susceptible to TK^D viruses than are adult mice. When adult mice were inoculated intracerebrally with 40 PFU of the parental clone (ACV-sensitive virus) and treated 5 h after infection with ara-A or ACV (100 mg/kg per day administered twice daily for 4 days), the mortality was significantly reduced (data not shown). However, mice inoculated with 4,000 PFU of G-ACV-C1 were essentially refractory to ACV treatment but responded to ara-A or a combination of ara-A and ACV (Table 1). At this dose, less than 10% of the mortality can be attributed to toxic effects of the drugs (12). Even at doses of 300 mg/kg per day (twice a day for 4 days), the mice were totally refractory to ACV treatment but responded to ara-A (100 to 300 mg/kg) or a combination of ara-A and ACV (100 or 300 mg of each per kg). These results clearly demonstrate that in this model, high doses of ACV could not overcome the pathogenic effects of this ACV-resistant variant and that a combination of ACV and ara-A is capable of preventing chronic disease and death.

Biron et al. (2) have shown that the ACV level in cerebrums of infected or uninfected mice after administration of a single dose of the drug (100 mg/kg) was between 10 and 50 μM for at least 4 h after inoculation and gradually dropped to about 1 μM after 24 h. In cell culture, these drug levels are adequate for total inhibition of most ACV-sensitive wild-type HSV-2 viruses (14). Hence, the lack of activity of

TABLE 1. Effect of ara-A and ACV alone and in combination in adult mice infected intracerebrally with an ACV-resistant HSV-2 variant (G-ACV-C1)

Treatment	Dose (mg/kg per day)[a]	PFU	Mean time to death ± SD (days)[b]	Mortality (no. of mice dead/no. treated [%])[b]
Phosphate-buffered saline		4		0/6 (0)
		40	10.0[c]	1/10 (10)
		400	6.5 ± 1.3	4/15 (27)
		1,000	10.5 ± 7.4	4/11 (36)
		4,000	5.4 ± 2.0	19/22 (86)
		40,000	2.3 ± 1.0	6/6 (100)
ACV	100	400		0/14 (0)
	100	1,000	5.7 ± 2.9	7/12 (58)
	100	4,000	6.3 ± 2.6	18/22 (82)
	200	4,000	8.1 ± 2.2	8/12 (67)
	300	4,000	4.7 ± 2.6	9/12 (75)
Ara-A	100	400	19.0	1/15 (7)
	100	1,000		0/12 (0)
	100	4,000	9.0	1/10 (10)[d]
ACV-ara-A	100/100	400	7.0	1/15 (7)
	100/100	1,000	6.0	1/12 (8)
	100/100	4,000		0/10 (0)[d]
Phosphate-buffered saline (no virus)				0/16 (0)

[a] Drugs were administered intraperitoneally twice daily for 4 days, beginning 5 h after intracerebral inoculation. The methods for inoculating and treating the animals have been described previously (14).

[b] Only animals which died on or before day 21 after virus inoculation are included.

[c] Single numbers indicate death of single animal.

[d] $P < 0.01$ that the observed increase in the number of survivors (Fisher's exact test) or the observed increase or decrease in the mean time to death (Mann-Whitney test) was due to chance when compared with the data for the corresponding untreated group.

multiple doses of ACV in mice infected with ACV-resistant HSV-2 cannot be attributed to suboptimal drug levels in the brain (Table 1).

There are few reports on the effectiveness of combinations of drugs in animals infected with drug-resistant variants (M. N. Ellis, D. C. Lobe, R. W. Morrison, and T. Spector, Abstr. Inter-Am. Soc. Chemother., p. 44, 1985). This may be due to the finding that most of these variants produce attenuated disease, making it difficult to determine a definite clinical response (5). However, in experimentally induced HSV encephalitis in mice, certain ACV-resistant HSV-2 TK^D variants can retain their virulence, and this model may provide useful information on the in vivo resistance and cross-resistance patterns of drug-resistant viruses. Although this model has provided accurate predictions of the clinical usefulness of various antiviral drugs (10, 13), the pathogenesis of HSV TK^D variants in mice may be different from that in humans. In addition, the size of the virus inoculum used in the mouse studies (Table 1) may have been larger than is necessary for induction of herpes encephalitis in humans.

Although ACV resistance could not be overcome in mice by high doses of ACV, this is not the case in vitro (11). This dichotomy between in vitro and in vivo drug resistance may be related to the greater toxicity of selective antiviral drugs in infected than uninfected cells.

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